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Michelle L. Buelow April 14, 2011

The Epidemiology of Rickettsial Diseases on the US Mexico Border: An Analysis of Incidence Rates, Clinical Presentation and Risk Factors Associated With *Rickettsia rickettsii, Rickettsia typhi* and *Ehrlichia chaffeensis* Infection

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Abstract

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By Michelle L. Buelow

Rickettsia rickettsii, Rickettsia typhi and *Ehrlichia chaffeensis* are three pathogens that can cause severe morbidity and mortality when not properly diagnosed and effectively treated. Because of their nonspecific clinical presentations, there are many challenges in their diagnosis, causing rickettsial diseases to be under-recognized and underreported. The goal of this study is to describe the burden and analyze epidemiological risk factors of rickettsial diseases in patients presenting with syndromic febrile illness between 2007 and 2008 in two South Texas counties. As a part of the Binational Infectious Disease Surveillance (BIDS) program, 1,392 patients with undifferentiated febrile illness were enrolled at surveillance sites in Cameron County and Webb County, Texas. Serological testing was conducted for the presence of antibodies and corresponding titer levels of *R. rickettsii, R. typhi* and *E. chaffeensis* and cases were classified according to standard CSTE protocols.[1, 2] We found that rickettsial diseases occurred with increased incidence at the U.S. Mexico border compared with statewide and nationwide estimates. Risk of infection with all three pathogens increased with exposure to the outdoors. However, none of the pathogens were significantly associated with multiple border crossings, the use of protective equipment (such as insect repellent or protective clothing) or a reported history of an insect bite. The study population in Cameron County was significantly more likely to be infected with *R. rickettsii* and *R. typhi* than those in Webb County. The risk for infection with both *R. rickettsii* and *E. chaffeensis* was significantly associated with time progression through the study period. Risk of infection with *R. typhi* was significantly associated with increasing age, while male gender was significantly protective for infection with *R. typhi*. This study demonstrates the importance of rickettsial diseases as emerging infections along the U.S. Mexico border and provides an increased understanding of their risk factors. It also validates the importance of continued binational collaboration in the BIDS project as a way to diagnose and implement public health interventions with the hope of improving the health of the unique and dynamic communities living in the U.S. Mexico border region.

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BACKGROUND/LITERATURE REVIEW

Morbidity, Mortality and Incidence of Rickettsial Diseases

Rickettsial diseases caused by infection with *Rickettsia rickettsii, Rickettsia typhi,* and *Ehrlichia chaffeensis* can cause severe illness and even death in otherwise healthy adults and children. Severe complications of tick-borne rickettsial disease caused by *R. rickettsii* and *E. chaffeensis* include prolonged fever, renal failure, disseminated intravascular coagulopathy (DIC), hemophagocytic syndrome, meningoencephalitis, adult respiratory distress syndrome and a toxic shock-like illness.[3, 4] *Rickettsia typhi,* which is flea-borne, has been associated with culture-negative endocarditis, splenic rupture, and focal neurological deficits such as hemiparesis or facial nerve palsy.[5] In addition to causing severe morbidity, these rickettsial diseases can cause mortality in otherwise healthy populations.

Rocky Mountain Spotted Fever (RMSF), caused by *R. rickettsii,* is the most commonly fatal tick-borne disease in the United States.[6, 7] Case fatality rates range from < 1% to over 10% in some case series; fatal outcome is highest in children under five, adults over 70 years of age, and among American Indians.[6, 8, 9] In older studies during the pre-antibiotic era, case fatality rates of 20-30% were not unexpected.[3, 6, 7] Case fatality rates for *E. chaffeensis* infection have been found to be 3%, while cases of *R. typhi* have a case fatality rate of up to 4%.[4, 10]

Rocky Mountain Spotted Fever (RMSF), caused by *R. rickettsii*, is a nationally reportable illness whose incidence has been surveyed throughout the United States. Cases of RMSF have been reported in 48 states, but 64% of these cases were reported from only five states: North Carolina, Oklahoma, Arkansas, Tennessee and Missouri.[6] The

estimated average annual incidence of RMSF in the U.S., based on surveillance from 2000-2007, increased from 1.7 cases per million persons in 2000 to a peak of 7.2 cases per million persons in 2005, with 7.0 cases per million reported in both 2006 and 2007.[6] National incidence increased during 2000 through 2007, from 1.7 in 2000 to a peak of RMSF and Human monocytic ehrlichiosis (HME), caused by *E. chaffeensis,* both have a reported incidence that has continued to increase in the past decades and it is now thought that these infections may be more common than previously recognized.[3, 11, 12]

HME, caused by *E. chaffeensis,* first became a nationally reportable disease in 1998 and the incidence has increased steadily since then, from 200 cases in 2000, to 961 cases in 2008 (CDC, unpublished data). The national incidence also increased similarly, from less than 1 case per million persons in 2000 to 3.4 cases per million persons in 2008 (CDC, unpublished data). HME is most frequently reported from the southeastern and south-central areas of the United States, and three states (Missouri, Oklahoma and Arkansas) account for 35% of all reported *E. chaffeensis* infections (CDC, unpublished data). However, the true burden of disease is likely much higher than the reported incidence given lack of clinician recognition and underreporting.[13]

Rickettsia typhi, the cause of murine typhus, is not a nationally reportable disease and thus has an unknown national incidence. In the U.S., murine typhus is endemic in the southern geographic regions of the country from California to Texas and is reportable in California, Texas and Hawaii.[14] In recent years, California reported 4-21 cases annually, Hawaii 5-6 cases annually, and Texas 9-72 cases annually.[14] Given that there is no nationally recognized standard case definition or reporting system for murine typhus, the national incidence is unknown and comparisons between states are difficult to interpret.

Classification of Rickettsial Diseases

The Rickettsiaceae family and the classification of its members is complex and has been challenged recently by gene sequencing and antigenic data. Originally the Rickettisaceae family was identified using non-specific phenotypic characteristics. The pathogens were gram-negative bacteria that were associated with arthropods and necessitated eukaryotic cells for growth.[15] In the past 20 years, however, genetic phylogeny, gene sequencing and antigenic data have challenged the original classifications. The Rickettsiaceae family, which includes the *Rickettsia*, *Ehrlichia*, *Anaplasma, Wolbachia*, and *Neorickettsia* genera, are intracellular alpha proteobacteria associated with eukaryotic hosts (arthropods or helminths).[15]

Genetic and antigenic data have resulted in three main divisions of the *Rickettsi*a genus: the spotted fever group rickettsiae, the typhus group rickettsiae and the scrub typhus group rickettsiae, all which are transmitted by various arthropods resulting in different diseases. The spotted fever group makes up most tick-borne rickettsial diseases, including *R. rickettsii,* the cause of Rocky Mountain spotted fever.[15] The typhus group is made up of *R. typhi* and *R. prowasakii*. *Rickettsia typhi* is the cause of murine typhus and is transmitted by rat and cat fleas, while *R. prowasakii* is the cause of epidemic typhus transmitted by the human body louse. [15] Lastly, the scrub typhus group is made up of *Orientia tsutsugamushi* alone, which is transmitted by tromiculid mites (chiggers).[15]

The originally classified *Ehrlichia* genus has also been reclassified into four genera, based on genetic relatedness. *Ehrlichia* and *Anaplasma* species are transmitted by ticks, *Neorickettsia* by helminths, and *Wolbachia* are transmitted by both arthropods and helminths.[15] *Ehrlichia chaffeensis*, the cause of HME, and *Anaplasma phagocytophilum,*

the cause of human granulocytic anaplasmosis (HGA), are transmitted by ticks.[15] *Neorickettsia sennetsu*, transmitted by trematodes, causes Sennetsu ehrlichiosis.[15] *Wolbachia*, a symbiont of human filarial worms, has been shown to play a major role in filariasis.[15]

The transmission, clinical presentation, diagnosis and treatment of *R. rickettsii*

Rickettsia rickettsii is transmitted to humans by several species of ticks that feed on small mammals, including dogs (Figure 1). The most common vectors are *Dermacentor variabilis* (the American dog tick) in the eastern and central United States and *D. andersoni* (the Rocky Mountain wood tick) in the western U.S.[3] Recently, the common brown dog tick (*Rhipicephalus sanguineus*), a tick species common throughout the world and which has been shown to be a vector of *R. rickettsii* in Central and South America, was found to transmit *R. rickettsii* in eastern Arizona.[16] Lastly, the cayenne tick (*Amblyomma cajennense*), commonly known to transmit RMSF in Central and South America, was found to be a vector of RMSF in Texas as well.[3] Dogs are also susceptible to infection with *R. rickettsii* and have been found to develop RMSF simultaneously with their owners.[17] The epidemiological distribution of RMSF varies by the geographic distribution and behaviors of their tick vectors and dog populations.[3]

Rickettsia rickettsii infects endothelial cells causing a small-vessel vasculitis and leading to non-specific symptoms and various clinical presentations. The clinical presentation of RMSF varies in severity depending on whether it causes a small-vessel vasculitis limited to the endothelial cells, or if it expands to cause vasculitis in major visceral organs**.** Most patients present within the first 2-4 days of illness, after an

incubation period of 5-10 days post- tick bite.[18] Initial symptoms are non-specific and similar to most benign viral illnesses, including a sudden onset of fever, chills and headache, as well as myalgia or malaise.[3] Photophobia may be common in adults with a severe headache.[3] Nausea, vomiting and anorexia may also be present in RMSF. Children often present with acute abdominal pain, altered mental status and conjunctival injection.[3] Other signs and symptoms described but less commonly observed include bilateral periorbital edema, edema of the dorsal hands and feet, as well as calf tenderness.[3]

As a result of the vasculitis, a rash and other end-organ complications can occur in patients infected with *R. rickettsii*. A maculopapular or petechial rash is common 2-4 days after onset of fever in the majority of patients.[3] The rash usually begins as small, blanching, pink macules on the ankles, wrists or forearms that evolve to maculopapules.[3] In over half of the cases, the rash evolves to a generalized maculopapular or petechial rash, including the palms and soles, but is limited in its spread to the face.[3] While the rash is classic in the diagnosis of RMSF, it may be completely absent or atypical in up to 20% of RMSF cases.[19] The classic spotted or petechial rash presenting 5-6 days into the illness demonstrates progression and severity of the disease to include vasculitis of major organs resulting in life-threatening complications such as: "prolonged fever, renal failure, disseminated intravascular coagulopathy (DIC), hemophagocytic syndrome, meningoencephalitis and acute respiratory distress syndrome".[3] Focal neurologic deficits, including cranial or peripheral nerve paralysis or sudden transient deafness may also be observed.[3]

The diagnosis of RMSF is based on clinical and laboratory evidence of infection. Clinically compatible evidence of infection with *R. rickettsii* requires a reported fever and

one or more of the following: rash, headache, myalgia, anemia, thrombocytopenia, or any hepatic transaminase elevation.[2] Laboratory evidence can include detection of *R. rickettsii* using polymerase chain reaction (PCR) and immunohistochemical methods (IHC) in skin biopsy specimens, as well as by PCR in whole blood specimens taken during the first week of illness, before any treatment with antibiotics.[2] Other laboratory evidence that is used more commonly for the diagnosis of RMSF includes serological studies of the antibody response using the indirect immunoflourescence antibody (IFA) assay or the enzyme-linked immunosorbent assay (ELISA).[3] While serological testing using IFA is the gold standard, it can be challenging.[3] First, patients may lack an antibody response during the acute phase of illness, the time period when most patients seek medical care. Secondly, a convalescent serum sample is essential to confirm infection, posing an additional challenge in obtaining the correctly timed specimen in a clinical setting.[3]

When administered quickly and accurately, the appropriate treatment and management of RMSF can decrease case fatality rates from 20% to less than 5%.[3, 18] Doxycycline is the treatment of choice in both adults and children and should be initiated as soon as RMSF is suspected.[3] Most patients respond to doxycycline within 24-48 hours after initiation of therapy; however, severely ill patients may require longer periods of therapy before clinical improvement is noted.[3] A delay in treatment can lead to severe complications and fatal outcomes, while the use of appropriate treatment has been shown to decrease fatal outcomes from 20% to 5%.[3, 18] Although doxycycline and other tetracyclines are generally contraindicated in pregnancy, they may be warranted when tick-borne rickettsial diseases cause life-threatening complications.[3] While appropriate

treatment improves outcomes, it is important to note that some patients with tick-borne rickettsial diseases may require hospitalization in order to more effectively manage organ dysfunction, severe thrombocytopenia, mental status change and other needs necessitating supportive therapy.[3] Given that an effective treatment for RMSF exists, it is important for clinicians to promptly recognize and diagnose the patient in order to appropriately treat and manage the illness and improve overall health outcomes.

The transmission, clinical presentations, diagnosis and treatment of *E. chaffeensis*

Erhlichia chaffeensis is similar ecologically to R. rickettsii and it is also transmitted to humans by ticks. The most common tick vector for E. chaffeensis is Amblyomma americanum (the lone star tick) that is hosted by the white-tailed deer (Figure 2).[3] This tick is the most prevalent tick in the southeastern United States and extends from the South Central states to New England states.[3]

E. chaffeensis infects circulating lymphocytes where they divide into host membrane-bound clusters called morulae, leading to non-specific symptoms and a clinical presentation that resembles RMSF. Initial symptoms include a sudden onset of fever, chills, headache, malaise and myalgia, a nearly identical presentation to RMSF.[16] The most common abnormalities seen on physical exam in patients with *E. chaffeensis* infection include fever, rash, headache, and hepatosplenomegaly[20] Children are more likely to report nausea, vomiting and anorexia, as well as abdominal pain, altered mental status and conjunctival injection.[3] Other less common findings described for *E. chaffeensis* infection include periorbital edema, edema of the dorsal hands and feet, nuchal rigidity, cervical or inguinal adenopathy and calf pain and tenderness.[3]

As a result of the lymphocytic infection, a rash and other end-organ complications can also occur in patients infected with *E. chaffeensis*. A rash occurs 36-47% of the time in adults, but in 66% of children infected with *E. chaffeensis*.[20] The rash associated with *E. chaffeensis* infection is commonly distributed on the trunk or extremities and varies from maculopapular, to petechial, diffuse erythema or a combination of all three.[3, 20] When comparing the rash that occurs in *E. chaffeensis* infection to the rash that occurs in RMSF, it typically occurs later in the course of the disease (5 days after symptom onset); however, they can be difficult to distinguish from each other.[3] Similarly, the end-organ complications and mortality that occurs in RMSF occurs in *E. chaffeensis* infection but with less frequency.[3]

The diagnosis of *E. chaffeensis* is also based on standard case definitions that include clinical and laboratory evidence of infection. Clinical evidence includes any reported fever and one or more of the following: headache, myalgia, anemia, leukopenia, thrombocytopenia, or any hepatic transaminase elevation.[1] Laboratory evidence includes the identification of morulae in the cytoplasm of monocytes or macrophages by microscopic exam or serological evidence of an antibody response to *E. chaffeensis* using immunofluorescence assay (IFA) or Enzyme-Linked Immunoassay (ELISA).[1] Other diagnostic methods include detection of *E. chaffeensis* DNA in a clinical specimen using PCR or the use of immunohistochemical methods to identify ehrlichial antigens in a biopsy.[1]

The appropriate treatment and management of *E. chaffeensis* is similar to that of RMSF. Doxycycline is the treatment of choice for adults and children and should be administered promptly to avoid progression of severe disease and fatal outcomes,

similarly to RMSF.[3] Management may also include hospitalization, as at least 50% of patients with *E. chaffeensis* infection are hospitalized to rule out other life-threatening conditions and provide appropriate medical therapies.[3] When managed appropriately, *E. chaffeensis* infection has decreased clinical severity and most patients recover without any long-term consequences.[3]

The transmission, clinical presentations, diagnosis and treatment of *R. typhi*

The ecology of *R. typhi* infection involves fleas and small mammals, usually rodents, before it is transmitted to humans, resulting in a varied clinical presentation of non-specific symptoms. *Rickettsia typhi* is transmitted to humans by several species of fleas that feed on small mammals, including rats, cats and opossums (Figure 3). The typical urban reservoirs include the roof and Norway rats (*Rattus rattus and R. norvegicus,* respectively), and the rat flea (*Xenopsylla cheopis*) is the vector.[5] In more suburban regions, domestic cats and opossums may also play a role in maintenance and transmission of *R. typhi* and fleas are the vector.[5] *Rickettsia typhi* multiplies in the epithelial cells of the flea's midgut and is shed in the feces. Humans become infected by flea bites as well as flea feces inoculating into the bite site.[5] Once *R. typhi* is transmitted to humans by the flea, they parasitize the endothelial cells and cause vasculitis, leading to non-specific symptoms and a variety of clinical presentations. The incubation period usually lasts 7-14 days, and the most common presenting symptoms include fever, headache, rash and arthralgia.[5] Other signs and symptoms of murine typhus that are less common include hepatosplenomegaly, cough, diarrhea, nausea/vomiting, abdominal pain and confusion.[5]

As a result of the vasculitis, a rash and other end-organ complications can also occur in patients with murine typhus. The presence of rash with *R. typhi* is variable and has been found in as few as 20%, and as many as 80% of murine typhus cases.[5] The rash lasts 1-4 days and is non-pruritic and macular or maculopapular in nature. It begins on the trunk about 1 week after the onset of fever and spreads peripherally, sparing the palms and soles.[5] If the vasculitis spreads to major organs, severe complications such as endocarditis, splenic rupture and serious neurologic deficits can result.[5]

The diagnosis and treatment of murine typhus is based on clinical and laboratory evidence of infection. Clinically compatible evidence is equivalent to clinical evidence for RMSF and includes any reported fever plus one or more of the following: rash, headache, myalgia, thrombocytopenia, or any hepatic transaminase elevation.[21]. The gold standard to diagnose *R. typhi* is IFA serological testing, necessitating a properly timed acute and convalescent specimen demonstrating a four-fold rise in titer levels.[5] Other diagnostic methods include PCR or isolation of *R. typhi* from blood cultures.[5] When diagnosed accurately, the appropriate treatment of murine typhus has not only been shown to decrease mortality rate, but also shortens the course of illness. Doxycycline is the preferred antibiotic for the treatment of murine typhus in both adults and children.[5] It has been found to shorten the course of febrile illness and also has brought the mortality rate of murine typhus down from 4% to 1%.[5]

Epidemiological Risk Factors of Rickettsial Diseases

The demographics of patients, including their age, race, and ethnicity have been previously identified as factors that increase risk for rickettsial diseases. In a national

surveillance report of RMSF where cases were defined based on clinical and serological evidence, adults 50-59 and 60-69 years of age had the highest incidence of all age groups, while children less than 5 years of age had the lowest incidence rate.[6] Cases of RMSF occurred mostly among whites (86.8%), followed by blacks (7.9%) and American Indians (3.9%).[6] Race specific incidence was the highest for American Indian (16.8 cases per million population), than those for white (4.4), black (2.6), and Asian/Pacific Islander (0.5) race groups.[6] Hispanic ethnicity was reported for 4.1% of the cases.[6] Slightly more males (56.9%) were reported than females.[6] Another surveillance study of 10,000 military personnel found similar results in that the seropositivity of Spotted Fever Group rickettsiosis (including *R. rickettsii*, *R. parkeri*) was higher among older subjects and males. However, this study found that black non-Hispanic individuals had increased incidence rather than American Indian or whites.[22] Surveillance studies of *E. chaffeensis* involving clinical signs and symptoms, as well as serological evidence, also found that the highest incidence of HME occurred among the ≥ 50-year-old age group and males (CDC, unpublished data). Limited literature on incidence of *R. typhi* by age and demographic exists. In a recent report of 33 confirmed cases of *R. typhi* in Austin Texas, the average age of cases was 39 years, with only 15% being less than 18 years of age; 56% were male, and 97% were white.[21] It is reported, however, that while most cases of murine typhus are reported among adults, children can constitute up to 75% of infections in some outbreaks.[10, 23]

The geographic distribution of both patients and vectors has been previously identified as a factor that increases the risk of rickettsial disease. The East South Central Region and the South Atlantic were two regions of the United States with the highest

average annual incidence of RMSF.[8] Five states (North Carolina, Tennessee, Oklahoma, Missouri and Arkansas) reported the highest average annual state incidence rates of RMSF during the surveillance period of 2000-2007.[6] In the military surveillance study, subjects who were from states with "above-average" incidence of RMSF had a seroprevalence of SFG rickettsiosis that was significantly higher than those from states with an average incidence or lower.[22] Geographically, the states with the highest average annual incidence of HME were Missouri, Oklahoma and Arkansas (CDC, unpublished data). When states are combined, the regions reporting the highest incidence of HME were the southeastern and south-central United States (CDC, unpublished data). When looking at geographic epidemiology for murine typhus, Texas and regions of Southern California have the highest prevalence in the United States; however new reports have demonstrated spreading distribution of the typhus vectors and reservoirs.[23, 24]

Seasonality or temporal factors have also been demonstrated to be factors in rickettsial disease, likely related to peak vector activity. In the 7 year national surveillance report spanning 2000 through 2007, the majority of RMSF cases reported illness onset during summer months when tick activity is at its peak.[6] In the large surveillance study of 10,000 military personnel, temporality or seasonality were not taken into account as risk factors for increased seroprevalence of SFG rickettsiosis.[22] The national surveillance study of *E. chaffeensis* demonstrated that the highest incidence of HME occurred between the months of June and July, likely correlating to the season for increased numbers of adult and nymphal lonestar ticks (the primary life stages of ticks that bite humans and transmit infections) (CDC, unpublished data). Murine typhus also follows a seasonal distribution based on the population of flea vectors, which propagate

most successfully in hot and dry environments.[5] In one study of suburban environments, where domestic cats and opossums are speculated to be reservoirs, cases were prevalent from April through June.[5] However, in urban environments, where rats are the reservoirs, most cases of murine typhus were reported throughout the late summer and fall.[5] Similarly, in a recent outbreak in Austin suspected to be related to opossum reservoirs, the cases were most prevalent in late summer and early fall.[21]

Other factors that have been found to increase the incidence of rickettsial diseases include human behaviors or activities that may increase their exposure to vectors. For example, participation in outdoor activities in areas with high grass or weeds during April through September has been found to increase the risk of tick bites.[25] This may include both occupational and recreational activities such as hiking, camping, fishing, etc. Additionally, those who spend time outdoors in areas where vectors are endemic may increase their exposure and risk for rickettsial infections.[3] The military surveillance study demonstrated that those subjects who were members of ground military (army and marine corps) were significantly more likely to be seropositive for RMSF than those members of non-ground (navy and air force) military groups, likely due to a greater exposure to ticks in ground combat assignments.[22] A history of national or international travel to an endemic area also increases the risk of rickettsial diseases, particularly when the patient may have participated in outdoor activities that increase exposure to ticks.[3] Despite the history of travel to an endemic area increasing the risk of rickettsial diseases, there is no literature to demonstrate migratory status as a potential risk factor for increased incidence.

Challenges of Passive Surveillance of Rickettsial Diseases

There are several challenges involved with passive surveillance of rickettsial diseases, leading to limitations in the understanding of the true burden of disease. The first clinical challenge is that rickettsial pathogens present as non-specific febrile illnesses that mimic other viral illnesses and are notoriously difficult for the clinician to diagnose.[3, 4] This can result in under-recognition and diagnoses by health care providers. Not only are there difficulties with clinical diagnosis of rickettsial pathogens, but there are substantial challenges with laboratory diagnosis as well. Specifically, rickettsial pathogens are difficult to detect and identify using standard laboratory techniques and require the use of non-traditional laboratory methods.[4] Laboratory diagnosis using serological testing requires the attainment of a properly timed serum sample, both in the acute and convalescent phases, resulting in significant obstacles in diagnosis.[4] For example, if the serum sample is obtained too early in the acute phase of illness (the time when most patients seek medical care), an antibody response will not be detected.[3] Additionally, obtaining a properly timed convalescent serum sample requires the patient to return to the clinic within a certain time frame for further laboratory work after he or she has likely already recovered.[4] Lastly, the occurrence of cross-reactivity between related organisms during serological testing, particularly between spotted fever and typhus group rickettsiae, can lead to inaccurate diagnoses and classification of rickettsial organisms.[4] As a result of these challenges in both clinical and laboratory diagnosis of rickettsial diseases, rickettsial diseases are under recognized and the true burden of disease is likely under-represented.

Rickettsial Diseases in Mexico

Rickettsial diseases in Mexico have been documented, but limited literature exists elucidating the extent of the problem. Thus far, antigenically related rickettsial diseases, including *R. rickettsii*, *R. felis*, *R. prowazekii*, *R. typhi*, and *R. parkeri,* have been identified in Mexico.[26] Humans live in close proximity to animals and animals such as opossums, rats, and mice commonly inhabit backyards and houses in Mexico.[27] Some reports suggest that rickettsiosis is an emerging disease in Mexico, although this perception may be influenced by the implementation of new surveillance systems and educational efforts to promote increased diagnoses and treatment of rickettsiosis.[27]

Rickettisa rickettsii was first recognized as a disease of northern Mexico in the 1940s but received little attention in Mexico since then.[28, 29] More recently, however, several febrile cases in the Mexican states of Yucatan and Jalisco that were clinically thought to be dengue fever were found to have antibodies to spotted fever rickettsiae. Five people had antibodies to *R. rickettsii* alone, while ten had antibodies reactive to both *R. rickettsii* and *R. akari,* another genetically related species.[29] Later, in 2006, a fatal case of *R. rickettsii* was described in a child in southwestern Mexico where this infection had not previously been recognized. It was suggested that this case might have been the result of the reemergence of *R. rickettsii* throughout Mexico and Latin America. However, others have attributed it to the new rickettsial surveillance program that correctly identified the disease.[28]

A large urban outbreak of RMSF was identified in northwestern Mexico in 2009. In this outbreak, 1280 probable cases of *R. rickettsii* were identified in Mexicali, Mexico, which directly borders Imperial County, California.[30] Of these cases, 251 were

serologically confirmed and 12 fatalities were recorded, of which 6 have been laboratory confirmed. *Rhipicephalus sanguineus* (the brown dog tick) was identified as the vector during entomological surveys of dogs in the area.[30] This outbreak was epidemiologically similar to a recent outbreak in Arizona where stray dogs infested with the brown dog tick were also identified as the reservoir of *R. rickettsii.*[31] In 1909, pioneer rickettsial researcher Howard Ricketts died of typhus while he was investigating an epidemic typhus outbreak in Mexico City in hopes of learning more about spotted fever.[32] *Rhipicephalus sanguineus* had been previously recognized in outbreaks of *R. rickettsii* in Mexico since the 1940s; however, it was not until the recent outbreak in Arizona where it was considered of epidemiological importance in the transmission of *R. rickettsii*.[31] Risk for *R. sanguineus* transmission of *R. rickettsia* has been attributed to poor hygiene environments favoring high levels of tick infestation and transmission, as well as the close peridomestic associations between humans, dogs, and ticks.[30,31] As a consequence of the Mexicali outbreak, rickettsial diseases are now included as a routine differential diagnosis for suspect dengue cases and *R. rickettsii* has been detected in other Mexico states of Sonora, South Baja California, and Hidalgo.[30]

Literature on both *R. typhi* and *E. chaffeensis* in Mexico is extremely limited.[27] A recent study of healthy adult blood donors in Mexico City demonstrated antibodies against *R. typhi* in 14% of the samples.[33] However, in the Yucatan state, no infection with *R. typhi* was identified despite identifying *R. rickettsii* and *R. felis*.[28] Similarly, no cases of human monocytic ehrlichiosis had been identified in Mexico until 1999, in which one case was identified.[34] However, it was indistinguishable whether it was caused by *E. chaffeensis* or other closely related organisms such as *Ehrlichia canis* or *Ehrlichia*

ewingii. Despite the limited data and literature on rickettsial diseases in Mexico, the data that does exist indicates that these diseases are becoming of increasing public health importance. Given the lack of knowledge surrounding the epidemiology and burden of rickettsial disease in Mexico, further research is warranted.

The US/Mexico Border Region

The United States-Mexico border region is geographically and demographically unique, and continues to undergo rapid expansion. The United States-México border region, as defined by the 1983 La Paz Agreement, consists of the land within 100 km (62.5 mi) on either side of the international boundary.[35] It stretches approximately 2,000 miles from the Southern tip of Texas to California and is made up of 4 U.S states, 6 Mexican states, 44 United States counties, 80 Mexican municipalities, and 15 pair of sister cities (Figure 4).[35] This region is home to approximately 12 million inhabitants, which is expected to double by the year 2025.[35] In fact, two of the most rapidly growing metropolitan areas in the United States- Laredo and McAllen- are part of the Texas-Mexico border region.[35] Despite being largely Hispanic, there is also a Native American population at the border region with approximately 25 different Native American nations.[35] The population at the U.S.-Mexico border faces tremendous issues of poverty, with twenty-one of the counties on the border being designated as economically distressed areas.[35] In the U.S. border counties, the 2000 census reported an average yearly income of $14,560 and found that only 25-35% were medically insured.[35]

The population at the US-Mexico border region is dynamic and migratory, which is

thought to contribute to an increased risk of some infectious diseases. According to the U.S. Immigration and Naturalization Service, in 2002, more than 190 million people entered the United States from Mexico through 24 official ports of entry.[36] People from both countries cross the border frequently to work, go to school, shop, and seek medical care, or visit family and friends. This large population movement, combined with a limited public health infrastructure and poor environmental conditions, contribute to increased incidence of certain infectious diseases.[37] The emergence and reemergence of vector-borne infectious diseases such as West Nile Virus, Dengue, *Rickettsia*, and *Ehrlichia* at the United States-Mexico border area is a public health issue of interest for both nations.[36] While the incidence of rickettsial diseases are unknown at the U.S-Mexico border region, rates of RMSF, HME and murine typhus have been reported previously in Texas. From 2000 to 2007, Texas reported an average annual incidence of 0.96 cases of RMSF per million people[6] In 2002, Texas reported 0.16 cases of HME per million people.[4, 8] Other reports indicated that 9-25 cases of murine typhus were recently reported annually in Texas.[5]

Cameron County (one focus area for this study) is the southern-most county in the state of Texas and its geographic location at the U.S.- Mexico border contributes to the demographic make-up, poverty and the health of this population (Figure 5). According to the U.S. Census Bureau's 2009 estimates, Cameron County had a total population of 396,371 people with a racial makeup of 96.8% White, 1.3% Black or African American, 0.7% American Indian or Alaska Native, 0.6% Asian, 0.1% Pacific Islander and and 0.5% from two or more races.[38] 86.6% of the population was of Hispanic or Latino ethnicity.[38] Persons under 5 years old made up 11.1% of the county population, 35.0% of

the population were persons under 18 years old, and those 65 years old and over made up 11.1% of the county population.[38] According to the 2000 U.S. census, there were 97,267 households in Cameron County with 3.40 persons per household and a median household income of $30,950 in 2008.[38] 33.5% of people in the county were below the federal poverty level in 2008.[38] Given the poverty of Cameron County, the population is at increased risk for infectious diseases, including Rickettsial diseases.

Webb County is another Texas county along the Mexico border (the second site of this study) and is the largest county by area in South Texas (Figure 6**)**. According to the U.S. Census Bureau's 2009 estimates, Webb County had a total population of 241,438 people with a racial makeup of 97.6% White, 0.8% Black or African American, 0.6% American Indian or Alaska Native, 0.6% Asian and 0.4% from two or more races.[39] 94.5% of the population was of Hispanic or Latino ethnicity.[39] Persons under 5 years old made up 12.6% of the county population, 37.7% of the population were persons under 18 years old, and those 65 years old and over made up 8.1% of the county population.[39] According to the 2000 U.S. census, there were 50,740 households in Webb County with 3.75 persons per household and a median household income of $36,537 in 2008.[39] 26.6% of people in the county were below the federal poverty level in 2008.[39] As a result of the poverty of Webb County, its residents are also at increased risk for Rickettsial and other infectious diseases. In order to address the health issues that exist at the US-Mexico border, it is necessary to monitor diseases in this high risk population so that they can be better diagnosed, managed, and prevented in the future.

Binational Infectious Disease Surveillance Program

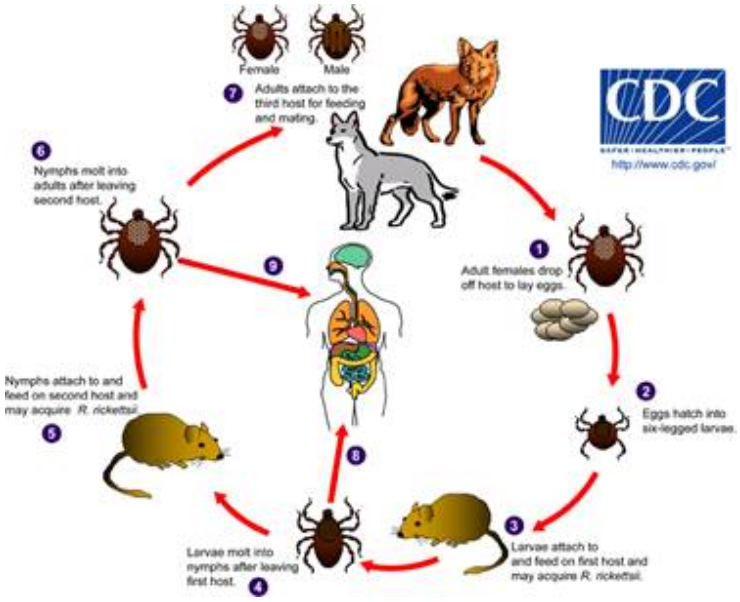
While surveillance systems have their limitations, they are necessary to better describe the epidemiology of diseases and gain more accurate diagnosis and prevention of severe illness and death. The Border Infectious Disease Surveillance project (BIDS) is a surveillance system that was established in order to better understand the burden of disease that exists at the US-Mexico border region. Given that infectious diseases remain impervious to the established geopolitical boundaries of the border, it became imperative to monitor infectious diseases in the border populations as one region rather than different populations on two sides of the border.[37] The Binational Infectious Disease Surveillance system (BIDS) was established in 1997 as an effort to bridge local, state and federal surveillance systems and form one binational surveillance system that enables the gathering of uniform epidemiologic data to improve disease control and prevention in the region.[37]

Since 1997, thirteen surveillance sites have been established in hospitals and clinics along the US-Mexico border region that serve as active sentinel surveillance sites for febrile exanthems.[37] The clinical sites are based in four sister cities and include four primary care clinics and three tertiary care hospitals in the U.S, as well as two general hospitals and four primary-care clinics in Mexico.[37] A standard protocol of laboratory testing was developed for patients who fulfill certain clinical criteria (*see Methods section for further details*). Patients who present with febrile exanthems are first tested locally for measles and rubella, and if found to be negative, the specimens are sent to a state or national reference laboratory to be tested for *R. rickettsia, R. typhi, E. chaffeensis*, and sometimes dengue virus. Serologic assays using standard protocols, techniques and

equipment were used in all laboratories in both the U.S. and Mexico. Active surveillance was conducted in order to enhance passive surveillance activities, which were then integrated into both state and national reporting systems.[37]

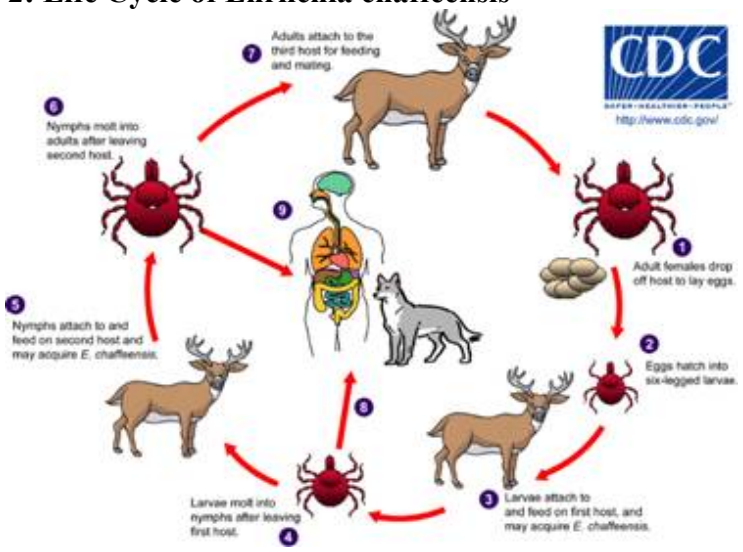
The syndromic surveillance of febrile exanthems will enable BIDS to better understand the magnitude of public health issues along the border. BIDS aims to determine the geographic distribution of diseases, detect outbreaks, monitor and evaluate control efforts such as vaccination programs, as well the monitoring of emerging infectious diseases.[37] Additionally, studying binational cases will contribute to the future understanding of mobile populations and their effects on infectious disease transmission.

Figure 1: Life Cycle of Rickettsia rickettsii



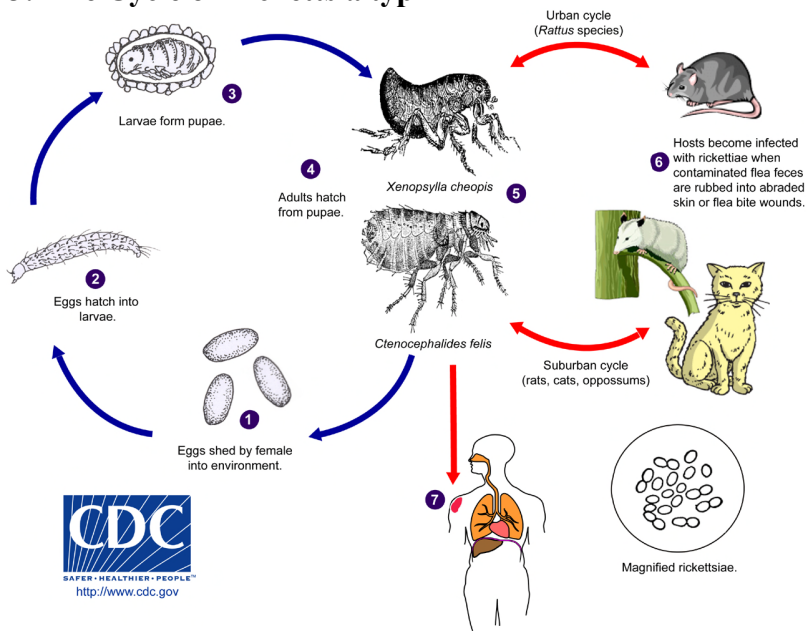
Source: Centers for Disease Control and Prevention (Accessed January 11, 2011)

Figure 2: Life Cycle of Ehrlichia chaffeensis



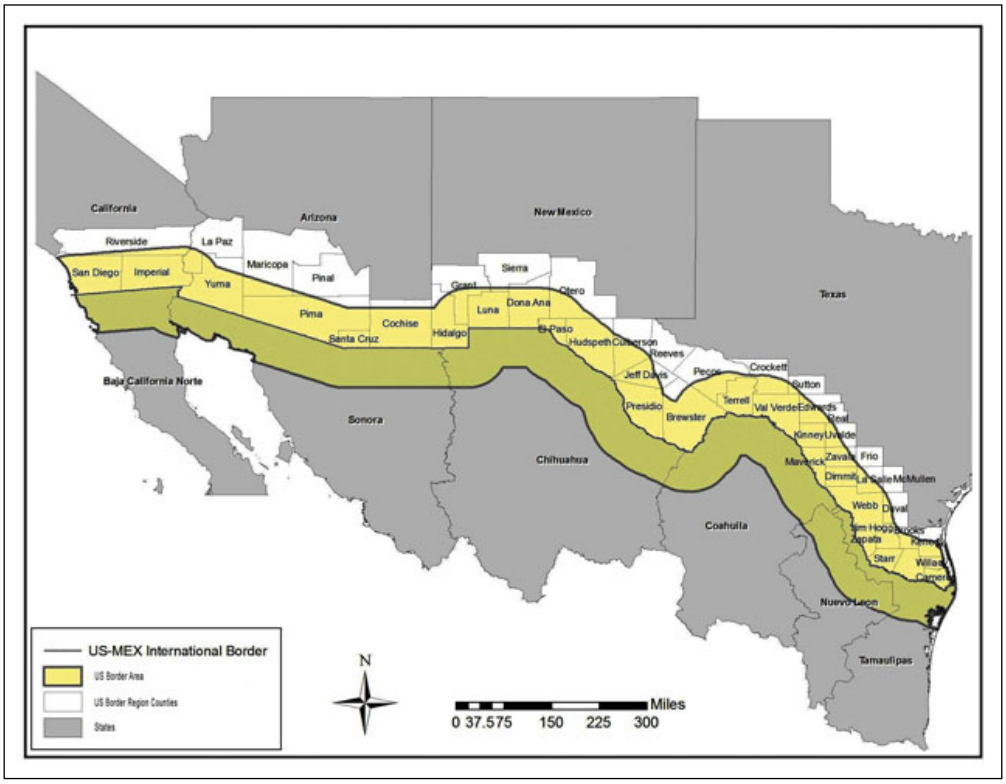
Source: Centers for Disease Control and Prevention (Accessed January 11, 2011)

Figure 3: Life Cycle of Rickettsia typhi



Source: Centers for Disease Control and Prevention (Accessed January 11, 2011)

Figure 4: United States-Mexico Border Region

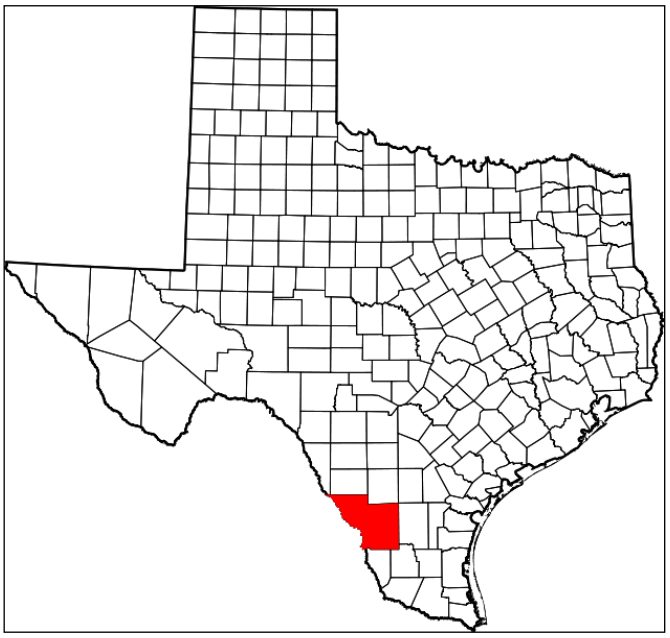


Source: United States-Mexico Border Health Commission: http://www.borderhealth.org/border_region.php. Accessed 1 Dec 2010.

Figure 5: Cameron County, Texas

**Red box outlines the geographic region of Cameron County*
Source: Centers for Disease Control and Prevention: http://www.cdc.gov/Pcd/issues/2008/oct/08_0106.htm. Accessed 29 Dec 2010.

Figure 6: Webb County, Texas



**Red area outlines the geographic region of Webb County*
Source: Wikimedia Commons.
http://commons.wikimedia.org/wiki/File:Map_of_Texas_highlighting_Webb_County.svg. Accessed 29 Dec 2010.

GOALS AND SIGNIFICANCE

Statement of Need

Because of their nonspecific clinical presentations, vector-borne illnesses are believed to be under-recognized and underreported at the U.S.-Mexico border, as well as the rest of the U.S.[3] In order to further prevent and control rickettsial disease, a better understanding and characterization of the burden and epidemiological risk factors for vector-borne illness is needed.

Goal

The goal of this project is to describe the burden and epidemiological risk factors of rickettsial diseases in patients with syndromic febrile illness between 2007 and 2008 in two South Texas counties.

Aims

1. To describe the signs and symptoms of probable cases of *Rickettsia rickettsii, Rickettsia typhi,* and *Ehrlichia chaffeensis* as well as the signs and symptoms of cases that were negative for all three Rickettsial diseases.
2. To quantify the rates of *Rickettsia rickettsii, Rickettsia typhi,* and *Ehrlichia chaffeensis* in febrile patients living in South Texas, specifically Cameron and Webb County, and compare them to the rates of the entire state of Texas.
3. To characterize the epidemiological risk factors, including demographic (age, race/ethnicity or gender), geographic (binational status) and temporal risk factors

(seasonality and exposure to tick or louse-bites) for infection by rickettsial pathogens at the U.S.-Mexico border.

a. Null Hypotheses to be Investigated:

i. There is no difference in age, race/ethnicity, gender, binational status or temporal risk factors among those who were classified as probable cases of *Rickettsia rickettsii* compared to those who were negative for all three rickettsial diseases.

ii. There is no difference in age, race/ethnicity, gender, binational status or temporal risk factors among those who were classified as probable cases of *Rickettsia typhi* compared to those who were negative for all three rickettsial diseases.

iii. There is no difference in age, race/ethnicity, gender, binational status or temporal risk factors among those who were classified as probable cases of *Ehrlichia chaffeensis* compared to those who were negative for all three rickettsial diseases.

Significance

In completing the above aims, this thesis will further elucidate the burden and epidemiology of three rickettsial diseases in two Texas counties. Given the increased socioeconomic risk factors of the border population, as well as their migratory characteristics, they are at an increased risk for emerging infectious diseases. Exploring the incidence of and risk factors for infection with these three rickettsial diseases will further our understanding of the evolving epidemiology of these vector-borne illnesses in the context of the U.S-Mexico Border. The knowledge gained about these three rickettsial

diseases can then be used to improve diagnosis and management of illness at the U.S-Mexico Border, while developing prevention strategies for similar populations at increased risk throughout the world.

METHODS

Study Setting

From January 1 2007 to October 31 2008, surveillance for undifferentiated febrile illness was conducted by the Texas Department of Health and Cameron County and Webb County Health Departments. In Cameron County, surveillance was conducted at eight sites in three different cities: Brownsville, Texas (Brownsville Community Health Center, Valley Regional Medical Center and Valley Baptist Medical Center–Brownsville), Harlingen (Harlingen Medical Center, Valley Baptist Medical Center–Harlingen, Regional Academic Health Center's Family Practice Residency Program and San Benito Medical Associates), and San Benito (Dolly Vinsant Memorial Hospital and San Benito Medical Associates).

In November 2006, surveillance started in Webb County at six sites in Laredo, Texas. City of Laredo Health Department (CLHD) conducted surveillance at the following sites: Laredo Medical Center, Doctors Hospital, Providence Surgical and Medical Hospital, Laredo Specialty Hospital, and Gateway Community Health Center.

At each surveillance site, health care providers were informed about the Binational Infectious Disease Surveillance (BIDS) project through medical executive committees, infection control committees, and meetings of emergency room physicians. Incentives for participation included the provision of cost-free laboratory diagnostics for any patient who fit the case criteria. Informational packets (that included a description of the project, case definitions, laboratory requisition forms, disease of interest fact sheets and list of notifiable conditions) were distributed. BIDS staff periodically contacted key

health care providers and personnel at each site to ensure proper logistical coordination and cooperation.

Sampling Method and Enrollment Criteria

The population under surveillance included all persons seeking medical care at participating sites who required specialized testing to establish a diagnosis and fit one of the additional enrollment criteria:

- Illness of greater than or equal to three days duration with fever and neurological symptoms or signs (e.g. headache, seizures, altered consciousness).
- Clinical diagnosis of viral encephalitis, meningoencephalitis, aseptic meningitis, acute flaccid paralysis, or atypical Guillain-Barre syndrome.
- Clinical diagnosis and/or laboratory test requested for West Nile infection, St. Louis encephalitis, dengue, arboviral infection, Rocky Mountain spotted fever, typhus, ehrlichiosis, or rickettsial infection.

Patients who were not enrolled prospectively could be enrolled retrospectively by BIDS staff who reviewed physician logs of hospital admissions, emergency room and outpatient visits and laboratory results. If patients met one of the three case criteria, their primary care physicians were contacted in order to obtain patient contact information and enroll them in the BIDS project.

Clinical information, including signs and symptoms, was obtained from providers or sites where patients were seen. Additionally, a standardized interview was conducted by the Sentinel Site Coordinator to obtain information on demographics, travel history

and outdoor exposures for each case. Data was not obtained for all variables; however, laboratory results and questionnaire data that were obtained were entered into a database and maintained by data managers at each site. Database entries were cross-checked by BIDS staff with patient medical records to ensure accuracy.

Given the nature of the surveillance data, analysis of data associated with this project was considered exempt from requiring IRB approval. All data collected through routine BIDS surveillance was de-identified prior to handling. Patient identifiers remained confidential throughout the collection and analysis processes.

Sample Collection

In patients who met the above case enrollment criteria, laboratory specimens were collected and shipped to the Texas Department of State Health Services (DSHS) laboratory in Austin. Standard IgG Immunoflourescence Assays (IFAs) were used and serological testing was conducted for the presence of antibodies and corresponding titer levels of *R. rickettsii, R. typhi* and *E. chaffeensis* per BIDS protocol. Acute serology was defined as serology drawn within 1 week of onset of illness. Convalescent serology was defined as serology drawn 2-4 weeks after onset of illness. Sentinel Site Coordinators distributed results to health care providers, infection control practitioner and hospital laboratories. Points of contact at each surveillance site were responsible for contacting patients to collect properly timed convalescent samples.

Data Analysis

Case classification was determined by the BIDS epidemiologists according to the below criteria and final case classifications were reviewed and confirmed by supervisors at the CDC.

Classification of Cases

Data were collected from a total of 1,392 patients. Cases were classified as probable, suspected, negative or insufficient according to the most recent CSTE case definitions for each pathogen and based on clinically compatible evidence and laboratory supportive evidence (Table 1).[1, 2] Clinically compatible illness of *R. rickettsii* and *R. typhi* infection was defined as fever with the presence of one or more of the following: rash, headache or myalgia. Clinically compatible illness of *E. chaffeensis* was one which included any reported fever and either headache and/or myalgia. Laboratory supportive evidence of *R. rickettsii, R. typhi* or *E. chaffeensis* was defined as elevated IgG antibodies with titer greater than or equal to 1:64. Supportive laboratory results were categorized according to four different laboratory conditions: 1) no acute specimen was available, but there was a positive convalescent serum, 2) a positive acute serum was paired with a positive convalescent serum, 3) the acute serum was negative, defined as a titer <1:64, paired with a positive convalescent serum or 4) there was a positive acute serum without a paired convalescent available.

Suspected cases were those that had no clinical information available but had laboratory supportive results. Negative cases were those for which a properly timed convalescent titer (taken at least 14 days after onset) was negative, defined as a titer < 1:64, whether or not clinically compatible evidence was present. Cases with insufficient evidence were those with clinically compatible evidence but with no acute or convalescent specimen was collected, or those with a negative acute titer but no convalescent titer was obtained. Probable cases required the presence of clinically compatible illness and laboratory evidence supportive of infection. The analysis of this thesis focused primarily on probable cases.

Binational Case Definition

Given the mission of BIDS to serve as a binational surveillance program, cases were also classified whether or not they were of binational relevance. A binational case, as defined by BIDS, was a 1) confirmed or probable case that, during the infectious period of the disease, traveled or lived in the other country or had contact with people who traveled or lived in the other country or 2) a suspected case that lived or traveled in the other country during the incubation period of the disease or 3) a case that requires the cooperation of both countries for investigation and control or 4) a case for which the history of the case implies a health risk for the other country.

Statistical Analysis

Data were collected by BIDS program staff and entered into the BIDS web-based database. Data were then imported into and analyzed in SAS v 9.2 (Cary, NC).

Many of the categorical variables were collapsed to form bivariate variables. Given that data for each variable were not reported in all cases, dichotomous comparisons were coded to reflect the instance in which a condition was reported and the instance in which a condition was not reported. Thus, both missing and negative responses were combined as a referent to positive responses. Tests of normality were conducted for the continuous variables visit date and age, neither of which were normally distributed. Wilcoxon Mann-Whitney tests were conducted for continuous variables and chi squared tests of association were conducted for categorical variables to identify the presence of statistically significant variation between cases of *R. rickettsii, R. typhi* and *E. chaffeensis* and non- cases, that tested negative for all three rickettsial pathogens. A p-value of less than 0.05 was considered statistically significant.

A multivariate logistic regression model was specified with county-specific fixed effects to identify risk factors and estimate adjusted odds ratios. All biologically and epidemiologically plausible variables were included in the model, including: age, gender, race, ethnicity, multiple border crossings, history of any insect bite, exposure to the outdoors, and the use of protective equipment (such as insect repellent or protective clothing). In order to control for potential non-response bias arising from missing values in epidemiologic variables, a dichotomous response variable was included in the model. Additionally, to identify any time trend or seasonality related to cases of illness, we included the continuous variable “time”, normalized from the date at which the case first presented in the clinic, as well as “time squared,” the square of the time variable, to allow for any possible nonlinearity in the relationship.

Table 1: Final Case Classification of Each Pathogen

	R. rickettsii **N (%)**	***R. typhi*** **N (%)**	***E. chaffeensis*** **N (%)**
Probable Case	48 (3)	78 (6)	31 (2)
Suspected Case	52 (4)	44 (3)	104 (8)
Negative Case	293 (21)	271 (19)	216 (17)
Insufficient Evidence	999 (72)	998 (72)	952 (73)
Total	**1392 (100)**	**1391 (100)**	**1303 (100)**

RESULTS

This study had three primary goals: 1) to describe the signs and symptoms of probable cases of *R. rickettsii, R. typhi,* and *E. chaffeensis*, as well as the signs and symptoms of cases that were negative for all three rickettsial diseases; 2) to quantify the incidence of *R. rickettsii, R. typhi,* and *E. chaffeensis* in febrile patients living in Cameron and Webb County Texas, and compare them to the rates of the entire state of Texas; and 3) to characterize the epidemiological risk factors for infection with these rickettsial pathogens at the U.S.-Mexico border.

Demographics

Over the entire surveillance period, from January 2007 through October 2008, 1,392 people met the BIDS enrollment criteria and became part of the study. Of these, 910 records were entered from Webb County, and 483 records were entered from Cameron County.

In order to better understand the study population, demographic information was collected from study subjects (Table 2). The study population had a median age of 28 years, a mean of 32.8 and an inter-quartile range of 8 and 53. Six hundred and twenty-one (48.7%) of the study population were male. 1,219 (87.6%) were Caucasian and 1,185 (94.2%) reported being of Hispanic or Latino ethnicity. The majority (82.8%) of the study population reported being born in the United States, and overwhelmingly, most of the cases (98.1%) did *not* meet BIDS criteria for being classified as a binational case. The study population was fairly young (with a median age of 28), predominantly female (51.3%), Caucasian (87.6%), and of Hispanic/Latino ethnicity (85.1%).

Clinical Presentation

In order to describe the clinical presentation of probable cases of *R. rickettsii*, *R. typhi*, and *E. chaffeensis*, data were collected from study subjects describing symptoms as well as clinical signs. Additionally, clinical data was collected from study subjects found to be negative for all three rickettsial diseases (Table 3). Fever was part of the case definition, and so was present for all cases. In the 48 probable cases of *R. rickettsii*, the three other most frequently reported symptoms were headache, muscle weakness and myalgia. Less frequently reported symptoms were arthralgias, stiff neck and retro-orbital pain. Rashes were reported in more than one third of the probable cases of *R. rickettisii*, predominantly a petechial purpuric rash. Other clinical signs that were frequently reported in probable cases of *R. rickettsii* included altered consciousness. Over half of the probable *R. rickettsii* cases were hospitalized but there were no reported deaths.

Of the 78 probable cases of *R. typhi*, the three most frequently reported symptoms other than fever were headache, myalgia and muscle weakness. Less frequently reported symptoms were arthralgias, stiff neck and retro-orbital pain. Almost half of the *R. typhi* cases reported a rash, mainly petechial purpuric rashes similar to cases of *R. rickettsii*. Other clinical signs that were reported in probable cases of *R. typhi* included altered consciousness. Over half of the cases were hospitalized but there were no reported deaths.

In the 31 probable cases of *E. chaffeensis*, the three most frequently reported symptoms in addition to fever were headache, muscle weakness and myalgia. Less frequently reported symptoms were arthralgias, retro-orbital pain and stiff neck. Probable

cases of *E. chaffeensis* reported a rash with less frequency than *R. rickettsii* and *R. typhi* However, when a rash was present, it tended to be described as a petechial purpuric rash similar to *R. rickettsii* and *R. typhi*. Other clinical signs that were reported in probable cases of *E. chaffeensis* included altered consciousness. Over half of the cases resulted in hospitalization, while none of the cases reported flaccid paralysis, shock, bleeding or death.

In the 190 cases that were found to be negative for all three rickettsial diseases, all of the reported symptoms, including muscle weakness, headache, stiff neck, arthralgias, myalgias and retro-orbital pain, were reported with less frequency. Negative cases reported a rash less frequently than each of the three pathogens. These cases were hospitalized with less frequency than the other three pathogens, but resulted in death with increased frequency.

In summary**,** cases of *R. rickettsii* and *R. typhi* most commonly reported headache, muscle weakness, and myalgia compared to other symptoms. *Rickettsia typhi* cases were more likely to report a rash than *R. rickettsii* and *E. chaffeensis.* A rash described as being petechial purpuric was most commonly reported. Cases of *E. chaffeensis* were more likely to report headache, muscle weakness and arthralgias than other symptoms and less likely to report a rash compared to *R. rickettsii* and *R. typhi*. Over half of the probable cases caused by each of the three pathogens led to hospitalization but no deaths were reported. In the negative cases, they were much less likely to report a rash and hospitalization, but reported deaths with increased frequency from cases. Thus, the presenting signs and symptoms of the probable cases varied between pathogens, as well when compared with negative cases.

Incidence Rates

In order to quantify the incidence of *R. rickettsii, R. typhi,* and *E. chaffeensis* in febrile patients living at the U.S- Mexico Border, we measured the rates of each of these rickettsial diseases in Cameron and Webb County Texas (Table 4). Rates were calculated from probable cases that were reported from January 1, 2007 through December 31, 2007. The denominator population that was used was based on the estimated 2007 U.S. Census data for each of the two counties.[38, 39] Given the estimated average national incidence rates of *R. rickettsii* and *E. chaffeensis* previously reported, the estimated incidence rates in Cameron and Webb Counties were comparatively much higher than the national incidence rates of each of the three pathogens; three to seven times higher for *R. rickettsii* and six to seven times higher for *E. chaffeensis*. However, the variation that exists in the prevalence of rickettsial disease by geographic and ecological location makes this comparison difficult. When using Texas as the comparison, however, the incidence rates of Cameron and Webb counties are still increased. Additionally, when comparing Cameron and Webb Counties to each other, Cameron County had an increased incidence of all three rickettsial diseases compared to Webb County. Given that *R. typhi* is not a nationally reportable disease, we are unable to compare incidence rates for this infection.

In summary, the study population of Cameron and Webb Counties at the U.S.-Mexico border have higher reported incidence rates of a *R. rickettsii* and *E. chaffeensis* than the state of Texas, as well as the national average.

Epidemiological Risk Factors- Unadjusted

Rickettsia rickettsii

In order to better understand the risk factors for infection with *R. rickettsii*, a bivariate analysis was conducted to identify potential risk factors, comparing probable cases to those in the study population that tested negative for all three diseases (Table 5). Factors that were non-significant included gender, crossing the U.S-Mexico border within 4 weeks prior to symptom onset, and having multiple border crossings (defined as at least one border crossing per week or more than one border crossing total in the 4 weeks prior to symptom onset). Reported Caucasian race and Hispanic/Latino ethnicity were found to be significantly protective for infection with *R. rickettsii*. Age was also significantly inversely associated with probability of being a case; as reported patient age increased, the risk of being a probable case decreased. The median age of *R. rickettsii* cases was 28 years (range 0-82), compared with the median age of those that were negative cases, which was 46 years (range 0-91; p-value 0.0139).

Other environmental factors that were found to significantly increase risk for infection with *R. rickettsii* included a history of any insect bite (including flea, tick, mosquito, lice, or an unknown) or any exposure to the outdoors, whether it be occupational or recreational. Furthermore, the use of protective equipment, such as insect repellent or protective clothing, was also found to be a significant risk for infection, as was having an air conditioning, window screen or swamp cooler where sleeping. When the relationship between case and time was examined, the median visit date for presenting as a case of *R. rickettsii* was October 6, 2007 (p-value <0.0001). As time progressed through the surveillance period, people were significantly less likely to

become a case of *R. rickettsii.*

In summary, factors that significantly increased risk of infection for *R. rickettsii* involved mostly environmental variables; behavioral factors such as border crossing did not significantly increase risk of infection.

Rickettsia typhi

To better understand the risk factors for infection with *R. typhi*, a bivariate analysis of potential risk factors was conducted which compared probable cases to those in the study population that tested negative for all three diseases (Table 5). Factors that were non-significant included gender, crossing the U.S-Mexico border within 4 weeks prior to symptom onset, and having multiple border crossings (defined as at least one border crossing per week or more than one border crossing total in the 4 weeks prior to symptom onset). Similar to *R. rickettsii,* reported Caucasian race and Hispanic/Latino ethnicity were also found to be significantly protective for infection with *R. typhi*. Additionally, age was significantly inversely associated with probability of being a case; as reported patient age increased, the risk of being a probable case decreased. The median age of *R. typhi* was 34 years of age (range 0-82), compared with the median age of those that were negative cases, which was 46 years (range 0-91; p-value 0.0191).

Other environmental factors that were found to significantly increase risk for infection with *R. typhi* included a history of any insect bite (including flea, tick, mosquito, lice, or an unknown) or any exposure to the outdoors, whether it be occupational or recreational. Furthermore, the use of protective equipment, such as insect repellent or protective clothing, was also found to be a significant risk for infection, as

was having an air conditioning, window screen or swamp cooler where sleeping. When the relationship between case and time was examined, the median visit date for *R. typhi* cases was August 12, 2007 (p-value <0.0001). As time progressed through the surveillance period, people were significantly less likely to become a case of *R. typhi*.

In summary, factors that significantly increased risk of infection with *R. typhi* were almost identical to those that increased risk of infection with *R. rickettsii* and involved mostly environmental variables; behavioral factors such as border crossing did not significantly increase risk of infection for either of the two pathogens.

Ehrlichia chaffeensis

In order to better understand the factors related to infection with *E. chaffeensis*, a bivariate analysis was conducted to identify potential risk factors comparing probable cases and negative cases (Table 5). Age was the only demographic variable that was a significant factor for *E. chaffeensis*, with the median age of cases at 26 years of age (range 2- 84), compared with the median age of those that were negative cases, which was 46 years (range 0-91; p-value 0.0037). As age increased, the risk of becoming a case decreased. Behavioral risk factors that were found to be significant included crossing the U.S-Mexico border within 4 weeks prior to symptom onset, and having multiple border crossings (defined as at least one border crossing per week or more than one border crossing total in the 4 weeks prior to symptom onset). Environmental factors that were found to significantly increase risk for infection with *E. chaffeensis* included a history of any insect bite (including flea, tick, mosquito, lice, or an unknown) or any exposure to the outdoors, whether it be occupational or recreational. Furthermore, the reported use of

protective equipment, such as insect repellent or protective clothing, was also found to be a significant risk for infection with *E. chaffeensis*, as was having an air conditioning/window screen or swamp cooler where sleeping. When examining seasonality for infection, the median visit date for first presenting as a case of *E. chaffeensis* was September 3, 2007 (p-value <0.0001). As time progressed through the surveillance period, people were significantly less likely to become a case of *E. chaffeensis*.

In summary, factors that significantly increased risk of infection for *E. chaffeensis* were similar to those of *R. rickettsii* and *R. typhi* and involved mostly environmental variables; however, behavioral factors such as border crossing also significantly increased risk of infection for *E. chaffeensis*.

Epidemiological Risk Factors- Adjusted

Rickettsia rickettsii

After adjusting for covariates using a multivariate logistic regression model with county-specific fixed effects, neither age, gender, race nor ethnicity was found to be significantly associated with infection with *R. rickettsii* (Table 6). Behavioral risk factors such as multiple border crossings and the use of protective equipment (insect repellent or protective clothing) both were not significantly associated with the disease; however, exposure to the outdoors significantly increased risk of infection with *R. rickettsii.* Having a history of an insect bite was not a significant predictor of infection. The relative risk of 7.8 for the county-specific fixed effect indicates that the study population of

Cameron County was nearly 8 times more likely than that of Webb County to become infected with *R. rickettsii.* However, the insignificance of time coupled with the significance of time squared indicated a slight nonlinear relationship, possibly seasonality, such that as time progressed through the study period, risk of infection increased slightly at a decreasing rate. This odds ratio (1.014) represents incremental change, as it is a continuous variable rather than dichotomous. Thus, an odds ratio of 1.014 means that the odds of being a case increased by 1.4% for each day beyond the mean visit date. In other words, at 30 days beyond the mean visit date, the odds of becoming a case are 42% higher if all else is equal. Specifically, cases were identified with slightly increasing frequency later in the study period, but this effect tapered toward the end of the study period.

Rickettsia typhi

After adjusting for covariates using a multivariate logistic regression analysis with county-specific fixed effects, the demographic variables race and ethnicity were not significantly associated with infection of *R. typhi* (Table 6). However, age was found to be a significant risk factor, while male gender was significantly protective for infection with *R. typhi.* Behavioral factors such as multiple border crossings or the use of protective equipment (such as insect repellent or protective clothing) were not significantly related to infection with *R. typhi*. However, exposure to outdoors significantly increased risk while having a history of any insect bite significantly decreased the risk infection with *R. typhi*. The study population in Cameron County was also significantly more likely to be infected with *R. typhi* than those in Webb County.

The insignificance of time as well as time squared demonstrated a lack of time trend and no detectable seasonality over the study period. There was no relationship between time and becoming a case of *R. typhi*.

Ehrlichia chaffeensis

After adjusting for covariates using a multivariate logistic regression analysis with county-specific fixed effects, neither age, gender, race nor ethnicity were significantly related to the risk of infection (Table 6). Multiple border crossings, a history of any insect bite, and the use of protective equipment were all insignificant in relation to infection with *E. chaffeensis*. Exposure to the outdoors significantly increased the risk of infection with *E. chaffeensis.* Unlike the previous rickettsial pathogens, the risk of infection with *E. chaffeensis* in our study population in Cameron County was not significantly different compared with that of Webb County. However, the insignificance of time coupled with the significance of time squared indicated a slight nonlinear relationship, possibly seasonality, such that as time progressed through the study period, risk of infection increased slightly at a decreasing rate. This odds ratio (1.021) represents incremental change, as it is a continuous variable rather than dichotomous. Thus, an odds ratio of 1.015 means that the odds of being a case increase by 1.5% for each day beyond the mean visit data. In other words, at 30 days beyond the mean visit date, the odds of becoming a case are 45% higher if all else is equal.

Table 2: Demographic Characteristics of Study Population

Variable	N (%)
Age *(N=1274)*	Median 28 IQR (8, 53)
Gender *(N=1275)*	
Male	621 (48.7)
Female	654 (51.3)
Race *(N=1392)*	
Caucasian	1219 (87.6)
African American	3 (0.22)
Other	170 (12.2)
Ethnicity *(N=1392)*	
Hispanic/Latino	1185 (85.1)
Non-Hispanic/Latino	207 (14.9)
Country of Birth *(N=209)*	
Mexico	36 (17.2)
U.S.	173 (82.8)
Binational Case *(N=1246)*	
Binational	23 (1.9)
Not Binational	1223 (98.1)

Table 3: Prevalence of Signs and Symptoms of Probable Cases by Pathogen

Sign or Symptom[a]	*R. rickettsii* (N = 48)		*R. typhi* (N= 78)		*E. chaffeensis* (N= 31)		All Negatives (N=190)	
	N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Fever	48	100.00	78	100.00	31	100.00	184	96.84
Headache	40	83.33	67	85.90	27	87.10	32	16.84
Hospitalized	30	62.50	51	65.38	18	58.06	57	30.00
Muscle Weakness	21	43.75	34	43.59	17	54.84	44	23.16
Myalgia	16	33.33	35	44.87	7	22.58	11	5.79
Petechial Purpuric Rash	14	29.17	25	32.05	3	9.68	8	4.21
Arthralgias	12	25.00	22	28.21	8	25.81	12	6.32
Retroorbital Pain	11	22.92	16	20.51	8	25.81	7	3.68
Stiff Neck	11	22.92	19	24.36	7	22.58	17	8.95
Altered Consciousness	4	8.33	8	10.26	3	9.68	5	2.63
Unknown Rash	2	4.17	3	3.85	0	0.00	1	0.53
Sensory Changes	1	2.08	5	6.41	2	6.45	4	2.11
Papular Rash	1	2.08	3	3.85	0	0.00	0	0.00
Seizures	1	2.08	0	0.00	2	6.45	5	2.63
Other Rash	1	2.08	3	3.85	0	0.00	1	0.53
Bleeding	1	2.08	2	2.56	0	0.00	3	1.58
Shock	0	0.00	1	1.28	0	0.00	1	0.53
Death	0	0.00	0	0.00	0	0.00	2	1.05
Flaccid Paralysis	0	0.00	0	0.00	0	0.00	1	0.53

[a] If sign or symptom is not present, it implies that patient did not report symptom or health care provider did not observe the symptom in the study population.

Table 4: Incidence Rates of Rickettsial Diseases in Webb and Cameron Counties in 2007 Compared to Reported National and Statewide Texas Incidence Rates

	Number of Cases per County:		County Incidence Rates (per 1 million)[a]		State of Texas Incidence Rates (per 1 million)	National Incidence Rates (per 1 million)
Pathogen:	**Cameron**	**Webb**	**Cameron**	**Webb**		
R. rickettsii	19	5	49.07	21.45	0.96[b]	7.0[b]
R. typhi	43	5	111.05	21.45	2.30[c]	Not Reported
E. chaffeensis	8	5	20.66	21.45	0.16[d]	3.4[d]

[a] *Based on estimated 2007 U.S. Census populations of Cameron and Webb Counties*[38, 39]

[b] *Rates of R. rickettsii are based on reported Texas and National Incidence Rates*[6]

[c] *R. typhi rates are estimate based on reported 48 cases per year reported (1990-2006) and 2000 U.S Census Estimates*[41, 42]

[d] *Rates of E. chaffeensis are based on reported Texas Incidence Rates in 2007*[4] *and reported National Incidence in 2008 (CDC, unpublished)*

Table 5: Unadjusted Bivariate Analysis of Probable Cases by Pathogen

	R. rickettsii			*R. typhi*			*E. chaffeensis*		
Variables[a]	**N**	**RR+**	**95% CI**	**N**	**RR+**	**95% CI**	**N**	**RR+**	**95% CI***
Time (based on Visit Date)+	238	0.995	**(0.993, 0.997)***	268	0.994	**(0.992, 0.996)***	221	0.995	**(0.993, 0.997)***
Age+	206	0.981	**(0.966, 0.996)***	235	0.985	**(0.974, 0.997)***	198	0.976	**(0.958, 0.993)***
Male	205	1.028	(0.550, 1.920)	234	0.802	(0.518, 1.242)	197	1.546	(0.731, 3.273)
Caucasian Race	238	0.404	**(0.246, 0.663)***	268	0.594	**(0.397, 0.886)***	221	0.559	(0.264, 1.186)
Hispanic/Latino Ethnicity	238	0.356	**(0.220, 0.574)***	268	0.535	**(0.368, 0.779)***	221	0.602	(0.283, 1.282)
Crossed Border in 4 wks prior to Symptom Onset	341	1.505	(0.760, 2.982)	349	1.502	(0.913, 2.473)	307	2.342	**(1.092, 5.021)***
Multiple Border Crossings	238	1.512	(0.690, 3.310)	268	1.534	(0.900, 2.614)	221	2.707	**(1.279, 5.726)***
History of Any Insect Bite[b]	238	2.635	**(1.620, 4.286)***	268	2.18	**(1.526, 3.114)***	221	3.592	**(1.932, 6.676)***
Exposure to Outdoors (Work or other Activities)	238	3.373	**(2.082, 5.467)***	268	3.104	**(2.169, 4.442)***	221	4.615	**(2.431, 8.763)***
Use of Protective Equipment (Repellant or Clothing)	238	3.849	**(2.454, 6.037)***	268	3.329	**(2.444, 4.533)***	221	5.063	**(2.812, 9.116)***
Window Screen or Air Conditioning Where Sleeps	238	5.175	**(3.005, 8.911)***	268	3.653	**(2.483, 5.374)***	221	7.541	**(3.568, 15.940)***
Swamp Cooler Where Sleeps	238	3.515	**(1.881, 6.569)***	268	2.708	**(1.734, 4.229)***	221	4.629	**(2.091, 10.247)***

[a] *Comparison group is negative for all 3 pathogens, defined as a convalescent titer < 1:68, 2-4 weeks after illness onset*

[b] *Reported insect bite includes that of a flea, tick, mosquito, lice, unknown or "other"*

**P-values < 0.05*

+ Odds ratios and CI reported for continuous variables (time and age) derived from logistic regression.

Table 6: Adjusted/Multivariate Analysis of Probable Cases by Pathogen

Variables[a]	*R. rickettsii* N = 205 RR	95% CI	*R. typhi* N = 234 RR	95% CI	*E. chaffeensis* N = 198 RR	95% CI
Age	1.020	(0.989, 1.052)	1.031	**(1.002, 1.061)***	1.021	(0.989, 1.053)
Male Sex	0.336	(0.095, 1.189)	0.353	**(0.125, 0.999)***	0.711	(0.213, 2.376)
Caucasian Race	0.980	(0.084, 11.383)	0.747	(0.123, 4.524)	1.029	(0.094, 11.297)
Hispanic/Latino Ethnicity	2.569	(0.342, 19.323)	2.217	(0.418, 11.757)	2.852	(0.243, 33.466)
Multiple Border Crossings	0.328	(0.043, 2.478)	1.055	(0.184, 6.059)	0.834	(0.156, 4.472)
History of Any Insect Bite[b]	0.818	(0.151, 4.435)	0.165	**(0.030, 0.902)***	0.357	(0.093, 2.194)
Exposure to Outdoors (Work or other Activities)	7.355	**(1.333, 40.582)***	18.305	**(3.293, 101.749)***	24.394	**(2.606, 102.937)***
Use of Protective Equipment (Repellant or Clothing)	6.319	(0.963, 41.460)	8.998	**(1.710, 47.359)***	4.472	(0.671, 21.217)
Cameron County (Vs. Webb)	18.539	**(2.798, 122.843)***	52.519	**(7.221, 381.958)***	5.848	(0.728, 36.082)
Time (based on visit date)	1.014	(0.998, 1.030)	1.008	(0.997, 1.020)	1.021	(0.998, 1.033)
Time Squared (based on visit date)	1.000	**(1.000, 1.000)***	1.000	(1.000, 1.000)	1.000	**(1.000, 1.000)***

[a] *Comparison group is negative for all 3 pathogens*

[b] *Reported insect bite includes that of a flea, tick, mosquito, lice, unknown or "other"*

[c] *P-value for this is 0.07(Reported CI crosses 1.0 and is not significant)*

**P-values < 0.05*

DISCUSSION

The goal of this study was to describe the burden and analyze the epidemiological risk factors for rickettsial diseases among patients presenting with syndromic febrile illness between 2007 and 2008 in two South Texas counties. Epidemiologically, exposure to the outdoors, being a case in Cameron County compared to Webb County and time progression were significantly associated with an increased risk of infection with *R. rickettsii.* In cases of *R. typhi*, age, exposure to outdoors and being a case in Cameron County compared to Webb County were found to significantly increase risk of infection. In cases of *E. chaffeensis,* exposure to the outdoors and time progression significantly increased the risk of infection. In conducting this study, we found that the incidence rates of rickettsial diseases among our study population were three to seven times that of the nationally reported incidence rates, and even higher compared to the incidence rates reported in the state of Texas.

Epidemiological Findings

Our findings of the epidemiological risk factors for infection with *R. rickettsii, R. typhi* and *E. chaffeensis* varied in their consistency with previously reported literature. Exposure to outdoors was associated with an increased risk for infection with *R. rickettsii, R. typhi* and *E. chaffeensis,* findings which have been documented previously as a significant risk factor for infection with all three pathogens[3,25]. Exposure to the outdoors likely increases risk of infection by increasing exposure vectors for these pathogens. However, fleas, which transmit *R. typhi*, are found in the peridomestic

environment and related to exposures to dogs, cats or opossums. Similarly, the brown dog tick, recently noted to have transmitted *R. rickettsii* in Arizona and Mexico, is closely linked to dog populations which are usually found in close proximity to humans. Thus, the significance of outdoor exposure may not be intrinsically related to outdoor activities so much as poor hygienic environments that promote animal-vector-human interactions.[16, 31]

Time progression, or temporality, was also found to significantly increase infection with *R. rickettsii* and *E. chaffeensis*. Despite seasonality being described as a risk factor in previous literature[6, 22], studying seasonality was not precisely possible in our study due to inconsistencies in the designated surveillance period. We attempted to use time (and time squared) based on date of illness onset as it progressed through the surveillance period as a proxy for seasonality. However, given that the peak number of cases for all three pathogens was inconsistent among all pathogens, and even varied with each pathogen between counties, there is a question whether or not this a systematic issue with surveillance as opposed to a biological finding. Of particular concern, the peak number of cases was not always in the summer months during peak tick activity, as it has been described in U.S. literature. For example, the peak number of *R. rickettsii* cases in Cameron County was found to be between November 2007 and January of 2008, winter months when tick activity should be decreased [data not shown]. Furthermore, we found the peak months of *E. chaffeensis* to be May through September [data not shown], while June and July are traditionally thought to be the peak months in the United States (CDC, unpublished data). There are two possible explanations for this: first is that the seasons of the border and Mexico do not correspond to the seasons traditionally described in U.S.

literature; for example, weather patterns along the border region may be more analogous to wet and dry seasons than the classic four season pattern observed in more northern U.S. climates. Secondly, time in our study may be correlated with increased surveillance efforts or changing patterns of case enrollment, and thus be an artifact of our surveillance systems rather than of true biological significance.

Our finding that there was a significant difference between cases of *R. rickettsii* and *R. typhi* who presented in Cameron County compared to those who presented in Webb County can be explained by two hypotheses. The first is that there could, in fact, be a biological difference that occurred between the populations that reside in each community and the risk factors that they were exposed to, or that regional variations in host and vector prevalence may actually impact disease transmission differently in the two counties. However, a more plausible explanation is that there was a systematic difference between the surveillance efforts employed in the two counties. For example, there may have been a difference in the staff who educated the health care providers regarding BIDS, or the physicians at each site who participated in BIDS. Therefore, providers at Cameron County may have better understood the role of BIDS and rickettsial diseases, and thus more actively screened patients who fit the enrollment criteria for than those at Webb County. As a result of a more active surveillance site, those patients who presented with febrile illness would be more likely to become enrolled in BIDS and tested for rickettsial disease.

Incidence Rates

Our study found elevated incidence rates of rickettsial diseases in Cameron and Webb Counties compared with those of national incidence surveillance. The national incidence rate takes into account geographic regions where rickettsial vectors are less prevalent. Comparing these incidence rates of Cameron and Webb Counties to the overall Texas rate is a more legitimate comparison, as we are comparing geographic regions with similar ecological make up and vector prevalence. However, when conducting these comparisons between the incidence rates of *R. rickettsii* and *E. chaffeensis* in our study population with those of Texas, the rates in Cameron and Webb counties are still increased.[3, 4, 6]

There are several possible explanations for the increased incidence rates of *R. rickettsii* and *E. chaffeensis* found in Cameron and Webb County compared to the state of Texas. The first possible explanation for increased incidence of *R. rickettsii* and *E. chaffeensis* in our study population is that there is known to be a persistence of antibodies to rickettsial diseases over time, and that even healthy persons living in enzootic areas may have antibodies due to prior exposures. The BIDS program may have captured some proportion of patient with febrile illnesses due to other causes but who had pre-existing antibodies to *R. rickettsii*, *R. typhi,* or *E. chaffeensis*. The concept of increased seroprevalence with age has been frequently suggested in rickettsial literature as the cause of increasing seroprevalence with an aging population.[3, 4]

A second possible explanation for increased incidence rates of rickettsial disease among our study population compared to the state of Texas is that it could be a result of

sampling bias. In general, rickettsial diseases are difficult to identify and diagnose due to their lack of specific clinical symptoms[3, 4]. On a national level, physicians are less likely to recognize these symptoms, diagnose and report rickettsial diseases, resulting in underreporting and an artificially low reported national incidence rate. However, given the BIDS program and physician education, patients who presented to surveillance sites with nonspecific symptoms were more likely to undergo a diagnostic work up for rickettsial illnesses than would otherwise undergo in a more routine clinical setting. This could result in an increased incidence rate among our study population that would have perhaps gone undetected at other clinical sites around the state of Texas and the country.

The last possible explanation for increased incidence rates of *R. rickettsii* and *E. chaffeensis* among our study population involves cross-reactivity of antigens that may result in similar antibody responses after infection with different rickettsial groups. Previous literature has reported on the cross reactivity between antibodies to *R. rickettsii* and *R.typhi,* as well as cross-reactivity between *E. chaffeensis* and other ehrlichial species.[3, 40] Thus, positive serologies for *R. rickettsii* in our study population may have resulted from previous infections with *R. typhi*, which is more prevalent in south Texas, or even due to other antigenically-related rickettsial species. This cross-reactivity is not well-understood but has been reported to impede epidemiological distinction between infections.

Our finding of increased *R. typhi* incidence compared to *R. rickettsii* and *E. chaffeensis* was an expected finding. *R. typhi* is enzootic in south Texas, meaning that it is endemic in the reservoirs and vectors of south Texas (as well as parts of California and Hawaii).[21] Recent studies in southern Texas and California have found that the classic

rodent-flea cycle of *R. typhi* has been augmented in suburban areas by the peridomestic cycle involving cats, dogs, opossums and their fleas.[21] As a result, our findings that the rates of *R. typhi* were much higher than that of the other two rickettsial pathogens are consistent with literature.

Strengths and Limitations

One of the key strengths of this study is that it reported on a unique surveillance program that studied three rickettsial pathogens simultaneously. This is the first study that we are aware of that tracks the epidemiology of all three of these rickettsial pathogens in the same patient population. This provides the opportunity to make comparisons across pathogens that would otherwise be made between separate and possibly dissimilar populations. A second strength of this study is that there are limited data on the U.S. Mexico border, despite knowledge that it is a high-risk population for emerging infectious diseases. This study provides insight into the epidemiology and burden of rickettsial diseases in a high-risk population. Furthermore, our approach was unique in that we used a multivariate model to look at epidemiological risk factors of all three pathogens at the same time.

One of the main limitations in this study was that the cooperation with the study varied between sites. Selection criteria for enrollment into the BIDS program were not applied stringently by physicians or BIDS staff. Additionally, there were several patients initially enrolled but for whom insufficient evidence was collected to classify the patient as a case. For example, not all patients received an interview to obtain clinical information, while others lacked a convalescent serum sample required to classify

probable cases. As a result, there was a large proportion of the study population who had to be excluded due to insufficient evidence to conclude if they were probable cases. Thus, a large sample of people who may have been cases of rickettsial infection went undetected, leading to underreporting and lack of understanding of the true burden of disease. Furthermore, of the cases that were identified, we are unable to determine whether the infection was acquired on the U.S. or Mexican side of the border. This information would have been valuable in order to implement effective prevention and detection strategies. Lastly, the results of our study are not generalizable as this was not conducted through random sampling methods, but was a passive surveillance system in a very specific population. Thus, the information that we gained from this study can only be applied to limited populations with similar characteristics as those living on the U.S. Mexico border.

CONCLUSIONS/ PUBLIC HEALTH IMPLICATIONS

It has previously been described that *R. rickettsii, R. typhi* and *E. chaffeensis* are three pathogens that can cause severe morbidity and mortality when not properly diagnosed and effectively treated. This study demonstrated the importance of rickettsial diseases as emerging infections along the U.S. Mexico border. In conducting this binational collaborative study:

- We demonstrated the challenges of diagnosing rickettsial diseases, and provided insight into the difficulties of surveillance and an explanation for underreporting.
- We found that despite these challenges, rickettsial diseases occurred with increased incidence at the U.S. Mexico border compared with statewide and nationwide estimates, providing a better understanding of the burden of rickettsial disease at the U.S. Mexico border.
- The demographic, behavioral and environmental risk factors that are thought to have contributed to increased rates of infection with *R. rickettsii, R. typhi* and *E. chaffeensis* were elucidated.

Given an increased understanding of the risk factors for infection with rickettsial diseases, the findings from this study may be useful to inform future prevention and treatment efforts for rickettsial infections along the U.S.-Mexico border. Specifically, it suggests that educational efforts for healthcare providers in this region should involve information on rickettsial disease. Providers should be informed of the need for empiric treatment with doxycycline in patients presenting with an illness clinically compatible

with rickettsial infections. Furthermore, providers should be aware of the need to collect both acute and convalescent specimens for serologic testing in order to further improve surveillance efforts.

The recent large-scale urban outbreak of Rocky Mountain Spotted Fever in Mexicali along the U.S.-Mexico border in 2009 demonstrates the potentially explosive nature of rickettsial outbreaks, with an accompanying high mortality. Enhanced surveillance systems focusing on high risk populations will be important in the future to identify areas of emerging concern and ensure opportunities for intervention. This study validates the importance of continued binational collaboration in the BIDS project as a way to diagnose and implement public health interventions, with the hope of improving the health of the unique and dynamic communities living in the U.S. Mexico border region.

REFERENCES

1. Ehrlichiosis and Anaplasmosis. *CSTE Position Statement Number: 09-ID-15* 2008; http://www.cdc.gov/ncphi/disss/nndss/casedef/Ehrlichiosis_2008.htm. Accessed Nov. 15, 2000.
2. Spotted Fever Rickettsiosis. *CSTE Position Statement Number: 09-ID-16* 2010. Accessed Nov 15, 2010.
3. Chapman AS, Bakken JS, Folk SM, et al. Diagnosis and management of tickborne rickettsial diseases: Rocky Mountain spotted fever, ehrlichioses, and anaplasmosis--United States: a practical guide for physicians and other health-care and public health professionals. *MMWR Recomm Rep.* Mar 31 2006;55(RR-4):1-27.
4. Demma LJ, Holman RC, McQuiston JH, Krebs JW, Swerdlow DL. Epidemiology of human ehrlichiosis and anaplasmosis in the United States, 2001-2002. *Am J Trop Med Hyg.* Aug 2005;73(2):400-409.
5. Civen R, Ngo V. Murine typhus: an unrecognized suburban vectorborne disease. *Clin Infect Dis.* Mar 15 2008;46(6):913-918.
6. Openshaw JJ, Swerdlow DL, Krebs JW, et al. Rocky mountain spotted fever in the United States, 2000-2007: interpreting contemporary increases in incidence. *Am J Trop Med Hyg.* Jul 2010;83(1):174-182.
7. Dalton MJ, Clarke MJ, Holman RC, et al. National surveillance for Rocky Mountain spotted fever, 1981-1992: epidemiologic summary and evaluation of risk factors for fatal outcome. *Am J Trop Med Hyg.* May 1995;52(5):405-413.
8. Chapman AS, Murphy SM, Demma LJ, et al. Rocky Mountain spotted fever in the United States, 1997-2002. *Vector Borne Zoonotic Dis.* Summer 2006;6(2):170-178.
9. Treadwell TA, Holman RC, Clarke MJ, Krebs JW, Paddock CD, Childs JE. Rocky Mountain spotted fever in the United States, 1993-1996. *Am J Trop Med Hyg.* Jul-Aug 2000;63(1-2):21-26.
10. Dumler JS, Taylor JP, Walker DH. Clinical and laboratory features of murine typhus in south Texas, 1980 through 1987. *JAMA.* Sep 11 1991;266(10):1365-1370.
11. Marshall GS, Jacobs RF, Schutze GE, et al. Ehrlichia chaffeensis seroprevalence among children in the southeast and south-central regions of the United States. *Arch Pediatr Adolesc Med.* Feb 2002;156(2):166-170.
12. Marshall GS, Stout GG, Jacobs RF, et al. Antibodies reactive to Rickettsia rickettsii among children living in the southeast and south central regions of the United States. *Arch Pediatr Adolesc Med.* May 2003;157(5):443-448.
13. Carpenter CF, Gandhi TK, Kong LK, et al. The incidence of ehrlichial and rickettsial infection in patients with unexplained fever and recent history of tick bite in central North Carolina. *J Infect Dis.* Sep 1999;180(3):900-903.
14. Wiggers RJ, Stewart RS. Ownership of cats or dogs does not increase exposure to Rickettsia typhi. *Tex Med.* Jun 2002;98(6):56-57.

15. Mendell. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases. 7th Ed ed: Elsevier Inc; 2007. Accessed Nov 28 2010.

16. Demma LJ, Traeger MS, Nicholson WL, et al. Rocky Mountain spotted fever from an unexpected tick vector in Arizona. *N Engl J Med.* Aug 11 2005;353(6):587-594.

17. Paddock CD, Brenner O, Vaid C, et al. Short report: concurrent Rocky Mountain spotted fever in a dog and its owner. *Am J Trop Med Hyg.* Feb 2002;66(2):197-199.

18. Thorner AR, Walker DH, Petri WA, Jr. Rocky mountain spotted fever. *Clin Infect Dis.* Dec 1998;27(6):1353-1359; quiz 1360.

19. Kirkland KB, Wilkinson WE, Sexton DJ. Therapeutic delay and mortality in cases of Rocky Mountain spotted fever. *Clin Infect Dis.* May 1995;20(5):1118-1121.

20. Jacobs RF, Schutze GE. Ehrlichiosis in children. *J Pediatr.* Aug 1997;131(2):184-192.

21. Adjemian J, Parks S, McElroy K, et al. Murine typhus in Austin, Texas, USA, 2008. *Emerg Infect Dis.* Mar 2010;16(3):412-417.

22. Graf PC, Chretien JP, Ung L, Gaydos JC, Richards AL. Prevalence of seropositivity to spotted fever group rickettsiae and Anaplasma phagocytophilum in a large, demographically diverse US sample. *Clin Infect Dis.* Jan 1 2008;46(1):70-77.

23. Whiteford SF, Taylor JP, Dumler JS. Clinical, laboratory, and epidemiologic features of murine typhus in 97 Texas children. *Arch Pediatr Adolesc Med.* Mar 2001;155(3):396-400.

24. Schriefer ME, Sacci JB, Jr., Dumler JS, Bullen MG, Azad AF. Identification of a novel rickettsial infection in a patient diagnosed with murine typhus. *J Clin Microbiol.* Apr 1994;32(4):949-954.

25. Walker DH. Rocky Mountain spotted fever: a seasonal alert. *Clin Infect Dis.* May 1995;20(5):1111-1117.

26. Zavala-Castro JE, Zavala-Velazquez JE, Peniche-Lara GF, Sulu Uicab JE. Human rickettsialpox, southeastern Mexico. *Emerg Infect Dis.* Oct 2009;15(10):1665-1667.

27. Zavala-Castro JE, Zavala-Velazquez JE, Sulu Uicab JE. Murine typhus in child, Yucatan, Mexico. *Emerg Infect Dis.* Jun 2009;15(6):972-974.

28. Zavala-Castro JE, Zavala-Velazquez JE, Walker DH, et al. Fatal human infection with Rickettsia rickettsii, Yucatan, Mexico. *Emerg Infect Dis.* Apr 2006;12(4):672-674.

29. Zavala-Velazquez JE, Yu XJ, Walker DH. Unrecognized spotted fever group rickettsiosis masquerading as dengue fever in Mexico. *Am J Trop Med Hyg.* Aug 1996;55(2):157-159.

30. Sanchez R AC, Lopez-Gatell H, Soria C, Estrada J, Olguin H, et al. Rhipicephalus sanguineus-associated Rocky Mountain spotted fever in Mexicali, Mexico: Observations from an outbreak in 2008-2009. Abstract #75. Paper presented at: The 23rd Meeting of the American Society for Rickettsiology 2009; Hilton Head, SC.

31. Eremeeva M, ML Zambrano and L Anaya, et al. Rickettsia rickettsii in Rhipicephalus Ticks, Mexicali, Mexico. *Journal of Medical Entomology*2011.

32. Price EG. *Fighting Rocky Mountain Spotted Fever in the Rockies*. Helena, MT: Naegele Printing Company; 1948.

33. Acuna-Soto R, Calderon-Romero L, Romero-Lopez D, Bravo-Lindoro A. Murine typhus in Mexico City. *Trans R Soc Trop Med Hyg.* Jan-Feb 2000;94(1):45.

34. Gongora-Biachi RA, Zavala-Velazquez J, Castro-Sansores CJ, Gonzalez-Martinez P. First case of human ehrlichiosis in Mexico. *Emerg Infect Dis.* May-Jun 1999;5(3):481.

35. United States-Mexico Border Health Commission. Border Region. 2010; http://www.borderhealth.org/border_region.php Accessed December 1, 2010.

36. Pan American Health Organization. United States- Mexico Border Area. *Health in the Americas* 2007; http://www.paho.org/hia/archivosvol2/paisesing/United%20States%20Mexico%20Border%20Area%20English.pdf.

37. Weinberg M, Waterman S, Lucas CA, et al. The U.S.-Mexico Border Infectious Disease Surveillance project: establishing bi-national border surveillance. *Emerg Infect Dis.* Jan 2003;9(1):97-102.

38. U.S. Census Bureau. Cameron County Census Quick Facts. 2000; http://quickfacts.census.gov/qfd/states/48/48061.html.

39. U.S. Census Bureau. Webb County Census Quick Facts. 2000; http://quickfacts.census.gov/qfd/states/48/48479.html.

40. Comer JA, Nicholson WL, Olson JG, Childs JE. Serologic testing for human granulocytic ehrlichiosis at a national referral center. *J Clin Microbiol.* Mar 1999;37(3):558-564.

41. Robinson LA. Murine Typhus in Texas2008:1-3. Located at: The EpiLink.

42. U.S. Census Bureau. Census 2000 Data for the State of Texas. 2000; http://www.census.gov/census2000/states/tx.html

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This form is to be used for students who have chosen to write a Literature Review or Special Project and are not required to apply for IRB approval.

Attach a one to two page description of the project including general subject, hypothesis to be tested or question(s) to be answered, and lay summary.

I have read the attached information and verify that this project is not research and therefore <u>does not</u> need to be submitted to the Emory University Institutional Review Board.

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Signature of Thesis Advisor **Date**